#25

PATENT **ATTORNEY DOCKET NO. VEOC.001-01US**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Geuze and Melief	)	Examiner: Amy DeCloux
	)	
Serial No.: 09/011,167	)	Art Unit: 1644
	)	
Filed: October 5, 1998	)	**APPELLANTS' BRIEF ON APPEAL**
	)	**SUBMITTED PURSUANT TO 37**
For: CELL DERIVED ANTIGEN PRESENTING VESICLES	)	**CFR § 1.192**
	)	

BOX AF
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

This Appeal Brief is submitted on or before Monday, November 19, 2001, the time period for response having been extended by the enclosed petition for extension of time and the requisite fee. Appellants hereby appeal from the final rejection of January 17, 2001 and the advisory action of May 4, 2001 finally rejecting claims 2-4, 6 and 13.

(1) Real Party of Interest

The Rijksuniversiteit te Leiden and the Universiteit Utrecht are the real parties of interest in the application at the time that the Brief is being filed.

2) Related Appeals and Interference

There are no related appeals nor interferences.

CERTIFICATE OF FIRST CLASS MAILING

I hereby certify that this paper or fee is being deposited with the United States Postal Service as first class mail in an envelope addressed to BOX AF, Assistant Commissioner of Patents and Trademarks, Washington, D.C. 20231

on November 19, 2001.

Signature: _____

Printed Name: Jeffrey M. Libby

(3) Status of Claims

There were submitted Claims 1 – 10 in the original application, Claims 11-13 were added by way of a preliminary amendment when the application entered national phase in the U.S.

Claim 1	Canceled
Claim 2	Pending
Claim 3	Pending
Claim 4	Pending
Claim 5	Canceled
Claim 6	Pending
Claim 7	Canceled
Claim 8	Canceled
Claim 9	Withdrawn from Consideration
Claim 10	Withdrawn from Consideration
Claim 11	Withdrawn from Consideration
Claim 12	Withdrawn from Consideration
Claim 13	Pending

(4) Status of Amendments

All of the pending claims, including Claims 2-4, 6 and 13, were finally rejected in the Office action dated January 17, 2001 (Paper 16). An Amendment After Final, filed April 17, 2001, in response to the final rejection was not entered by the Examiner because, as stated in the Advisory Action mailed May 4, 2001 (Paper 18), newly submitted Claim 14 would raise new issues requiring a new search. These same claim amendments, but without new Claim 14, were re-filed on July 17, 2001. This time, in the Advisory Action mailed August 17, 2001 (Paper 23), they were not entered because they would raise new issues and "Applicants have amended Claims 9, 11-12 to become product by process claims and therefore part of the elected invention is a

second after-final amendment when said amendments could have been made earlier". Claims 2-4, 6 and 13 are on appeal.

The text of the claims on appeal is provided in Appendix A. The text of the claims as they would be after entry of the attached proposed amendment is provided in Appendix B. Prior to review on appeal, Appellants respectfully request that the Examiner enter this amendment. The individual amendments were previously requested twice by Appellants. Entry of the amendment to Claim 13 will simplify the issues for appeal. The amendments to Claims 9 and 11-12 place the subject matter of the claims within the elected subject matter. There was no indication in Paper 18 that there was an issue relating to these claims, and the failure to have them entered upon re-submission (Advisory Action, Paper 23) for failing to have presented them previously is erroneous. Accordingly, Appellants respectfully request that they be entered at this time.

(5) Summary of the Invention

Claims 2-4, 6 and 13 (amended as proposed) are directed to an antigen presenting vesicle obtainable from an antigen presenting cell and free from its natural surroundings, comprising an MHC class I protein or a functional derivative or fragment thereof.

Claim 2 is supported by Claim 2 as filed, at page 2, lines 14 to page 4, line 3, on page 9, lines 11-20, and at page 5, line 35 through page 6, line 1.

Claim 3 is supported by Claim 3 as filed, and at page 6, lines 3-6 and page 6, lines 25-32.

Claim 4 is supported by Claim 4 as filed, and at page 6, lines 3-6 and page 6, lines 25-32.

Claim 6 is supported by Claim 6 as filed, and at page 6, line 1-2.

Claims 9 - 12 were withdrawn from consideration.

Claim 13 is supported by Claims 1 and 2 as filed, and at page 5, line 35 through page 6, line 1.

(6) Issue

Whether Claims 2-4, 6 and 13 are unpatentable under 35 U.S.C. § 112, first paragraph as being based on a non-enabling disclosure?

7) Grouping of Claims

Each of Claims 2-4, 6, and 13 are separately patentable and thus do not stand or fall together. Reasons why these claims do not stand or fall together are provided in the arguments that follow.

(8) Argument

Entry of Amendments

Appellants respectfully request the Board to enter the attached Amendment.

35 U.S.C. § 112, First Paragraph Rejection

A. The Examiner erred in that she did not explain why the exemplified procedures for isolation of Class II MHC vesicles could not be applied to isolation of Class I MHC vesicles.[1]

In order to satisfy the enablement rejection of section 112, a patent application must contain a description that enables one skilled in the art to make and use the claimed invention. Atlas Powder Co. v. E.I. DuPont De Nemours & Co., 750 F.2d 1569, 1576, 224 USPQ 409, 413 (Fed. Cir. 1984). When a patent specification contains a teaching of a manner and process of making and using the invention in terms that correspond in scope to those used in defining the claimed subject matter, it is presumptively enabling, whether it sets forth that teaching "by the use of illustrative examples *or by broad terminology*." (emphasis added) *In re Marzocchi* 169 USPQ 367, 369 (C.C.P.A. 1971). To overcome that presumption, the Examiner must set forth reasons to doubt the objective truth of the statements contained therein; i.e., the Examiner must explain why the accuracy of a statement is doubted and support that explanation with acceptable evidence. The Examiner has erred in that she did not provide acceptable evidence for finding that the claims are not supported by an enabling disclosure. The following is the evidence that was provided by the Examiner.

[1] In order to assist the Board, Appellants have attached hereto a Glossary of Terms as Appendix C.

> "There is insufficient guidance in the instant specification and in the prior art for an antigen presenting vesicle free from its natural surroundings obtainable from an antigen presenting cell comprising a membrane and an MHC Class I protein or fragment thereof, as recited in claim 13, and its dependent claims 2-4 and 6, though the specification, is enabling for an antigen presenting vesicle free from its natural surroundings obtainable from an antigen presenting cell comprising a membrane and an MHC class II protein or fragment thereof. As evidenced by Figure 1 in Delves et al., (Molecular Medicine Today, 3(2):55-60, 1997), it well known in the art that MHC Class I and Class II antigen presentation follow distinct, compartmentalized pathways, and that Class II bind antigens in endosomes or specialized loading compartments that are distinct from Class I molecules. Therefore, it is not clear from the instant specification that it is possible to isolate exosomes comprising class I (as opposed to class II) molecules from the supernatant of antigen presenting cells as taught by the specification." Emphasis in the original, Paper 16, page 3.

This evidence is insufficient because whether or not there are distinct compartmentalized pathways is irrelevant to methods for isolating vesicles using sucrose density gradients. Identifying where the amount of centrifugal force needed to pellet the organelles and where the two types of membranes would band in a sucrose density gradient would hardly require undue experimentation, and certainly has nothing to do with the reasons presented. Therefore, the Examiner has not provided acceptable evidence for doubting Appellants' teaching that the same methodology can be used for isolating both MHC class I and class II presenting vesicles from cells. The simple truth is, the same methodology may indeed be used, as further addressed below.

In the advisory action of May 4, 2001 (Paper 18), the Examiner expanded the rejection to include that Appellants had not taught "using" and additionally had not defined "vesicle":

> "[T]he examiner notes that said guidance regarding the making and using of said exosomes is disclosed in the context of class II only, not in the context of Class I. As repeated from the previous final rejection mailed 1-17-01, the examiner also agrees that the instant specification have (sic) described methods of differential centrifugation and

> isolation of subcellular fractions over sucrose gradients, but the examiner notes that the examples of an antigen presenting vesicle in the instant specification asserted by applicant all refer to the isolation of MIIC and exosomes. In view of the virtual absence of guidance from the instant specification of which vesicles contain class I proteins, and in view of the lack of sufficient guidance in the instant specification regarding how to make and use said vesicles comprising class I proteins, and in view of the lack of predictability concerning MHC Class I expression on exosomes as demonstrated by the post-filing date Zitvogel reference, and in view of the absence of a working definition of "vesicle" in the instant specification, the examiner maintains it would require an undue amount of experimentation on the part of one skilled in the art to make and use the claimed antigen presenting vesicle comprising a MHC Class I protein for the asserted utilities." Paper 18, page 1.

Setting aside the fact that new issues are raised by this rejection, and that the term "vesicle" is not only a well-known meaning in the art, but is also defined in the specification, there is still no explanation from the Examiner as to why one of skill in the art would require undue experimentation to use the simple technique of differential centrifugation and isolation of subcellular fractions over sucrose gradients as disclosed in the specification and well known to those of skill in the art to obtain MHC class I vesicles or why using the vesicles so obtained as a vaccine, for example, would require undue experimentation.

B. The techniques described for isolation of MHC class I vesicles as exemplified by isolation of MHC class II vesicles would not require undue experimentation.

"The test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent [application] coupled with information known in the art without undue experimentation." *United States v. Telectronics, Inc.*, 8 USPQ 2d 1217 (Fed. Cir. 1988). In *In re Wands* (8 USPQ 2d 100, 1404 (Fed. Cir. 1988) it is suggested that the following factors should be considered in determining whether a disclosure requires undue experimentation.

(i) The quantity of experimentation necessary

The methods required to produce the present invention, that is, growing antigen presenting cells in cell culture media, subjecting culture supernatants or lysates to differential centrifugation, following by linear sucrose density gradients, and analysis with Western blotting or antibody binding, require minimal, if any, experimentation. These techniques can be performed by technicians in cellular immunology or microbiology laboratories with readily available starting materials using methods that are well known and used every day in the art.

The specification teaches that MHC class I molecules are present on lymphocyte multivesicular intracellular compartments bearing MHC class II molecules (MIICs). The Zitvogel publication (Nature Med 1998, 4: 594-600), first cited by the Examiner, shows that differential centrifugation and fractionation over a linear sucrose gradient, *that was first disclosed in the instant specification*, may be used to isolate secreted vesicles (exosomes) that comprise MHC I and/or MHC II molecules. Western blot and immunoprecipitation analyses to detect the presence of MHC I molecules as well as MHC II molecules can be conducted using techniques well known to those in the art at the time of filing of the application, and by following the procedures taught in the instant specification (page 3, line 1 through page 4, line 11; page 8, line 4 through page 10, line 9; and page 12, line 31 through page 13, line 13 of the original specification), but using anti-MHC class I antibodies readily available to those in the art (see Zitvogel *et al.*, as well as Scott et al. (1995) *J. Immunol.* 155:143-148; Kaufman, *et al* (1995) *Proc. Natl. Acad. Sci.* 92:6484-6488; Atta, *et al* (1995) *Clin. Exp. Immunol.* 101:121-126; Khilko, *et al* (1995) *J. Immunol. Methods* 183:77-94; Rangel, *et al* (1995) *Eur. Cytokine. Net* 6:195-202, cited by Appellants on April 17, 2001 in their response to the final rejection). The work of Zitvogel, *et al* demonstrates the correctness of Appellants' assertion that "the exosomes possess MHC II and/or MHC I molecules at their surface", that the detailed differential centrifugation and fractionation procedures taught in the instant specification may be used to produce vesicles that comprise MHC I and/or MHC II molecules from antigen presenting cells, and confirms that undue experimentation is not required.

(ii) The amount of direction or guidance presented

As noted in the present specification, the disclosed vesicles will contain major histocompatibility complex I and/or II (page 6, lines 3-6 and Claims 2 and 4 as filed). Guidance is also provided on page 8, line 23 through page 9, line 8 for MHC class I in view of page 6, lines 3-6, disclosing MHC class I-containing vesicles, and Claims 2, 4 and 9 as filed, directed to MHC class I-containing vesicles and differential centrifugation of lysates or supernatants for producing MHC class I-containing vesicles.

One may readily isolate exosomes comprising class I molecules from the supernatant of antigen presenting cells as taught by the specification. For example, lines 2-3 of the Abstract state that "[e]xosomes are vesicles derived from MHC class II enriched compartments in antigen presenting cells. The exosomes possess MHC II and/or MHC I molecules at their surface and possibly peptides derived from processed antigens in said MHC's." Methods for the preparation of secreted vesicles were known at the time this present application was filed (see the references cited in the present application: ref. 5: Peters, et al. (1991), ref. 6, Riberdy, et al. (1994) and ref. 13, Harding, et al. (1984) and Pan, (1985)). The skilled artisan knows that class I MHC molecules are constitutively expressed in almost all nucleated cells of the body, including immune cells. It is also well known that lymphocytes, such as a human B cell line used to generate vesicles in the present invention, generally express higher levels of MHC class I proteins than any other cell in the body, which, in part, explains their presence on lymphocyte-derived vesicles. Thus, in view of the specification and the body of knowledge available in the art, the skilled artisan, rather than doubting the objective truth of the statements in the specification, would recognize that the disclosed methods for preparation of vesicles from antigen presenting cell would yield vesicles with MHC class I and/or II molecules on their surfaces.

As stated in the response filed July 17, 2001, **Methods for using vesicles comprising MHC class I protein** are provided and are known to those skilled in the art. The specification teaches how to use the claimed vesicles on Line 3 of page 7, which

refers to "the ... foremost use of these vesicles", in regard to the preceding lines comprising "MHC I or II" and vesicles "with desired peptides having the right binding motiv (sic) to fit in the respective MHC" (emphasis added). Uses for these vesicles include vaccines designed "to elicit an immune response against any proteinacious substance which has peptide antigens that can be presented in the context of MHC", and which may comprise "suitable adjuvants, if necessary, carriers, if necessary, ecxipients (sic) for administration, etc." (page 7, lines 6-13). Page 7, lines 14 –19 states "The vaccines can be used in the prophylaxis of many disorders, such as infections, immune disorders, malignancies, etc." "Very important applications will of course be the treatment or prophylaxis of AIDS, eliciting immuneresponses (sic) agains (sic) tumours and the like" (page 7, lines 14 – 19). Additional applications include "that [the vesicles] may be used to induce tolerance to certain antigens, for instance, by giving large doses of the vesicles orally" (page 7, lines 20-23). These annotations refer not only to MHC class II vesicles but also specifically to MHC class I vesicles (see page 6, line 3 through page 7, line 2).

Methods for using vesicles comprising MHC class I protein are also provided. The present application demonstrates how to use antigen-presenting vesicles to stimulate T cells (see legend to Figure 4, page 10, lines 12-27 of the original specification) in the context of MHC class II vesicles, but the procedure is the same regardless of the antigen source, as is well known to those of skill in the art. Routine experimentation would allow one to adjust the number of cells used as the source of vesicles and vaccines. These constitute reasons why "the presentation of peptides as antigens, for the stimulation of for instance T-cells" (page 7, lines 5-6) would be successful with vesicles prepared for said stimulation comprising "MHC I and II" (page 6, line 34) and "desired peptides having the right binding motiv (sic) to fit in the respective MHC" (page 7, lines 1-2).

Zitvogel *et al.*, have demonstrated the operability of using MHC class I bearing vesicles for stimulating T cells and for treatment of cancer. "To assess the capacity of DC-derived exosomes to induce effective T cell-mediated immune responses *in vivo*",

Zitvogel "evaluated their antitumor effects in tumor-bearing mice"(page 595, column 1, lines 33-35).

(iii) The presence or absence of working examples

Four working examples are provided by the specification for vesicles bearing MHC class II molecules on their surfaces (pages 8-10). The specification also indicates that vesicles with either one or both of MHC class I and MHC class II molecules are produced by the described methods from appropriate antigen presenting cells (page 6, lines 3-6): "[t]hese vesicles preferably will contain major histocompatibility complex (MHC) I and/or II...". In other words, these methods can be used as working examples to produce vesicles with MHC class I molecules on their surfaces. Other methods of producing vesicles are also described, such as synthetically-prepared liposomes (page 6, lines 25-29) and by recombinant means (page 6, lines 29-32). Support for making antigen-presenting vesicles is found in page 2, line 25 through page 3, line 7 and page 8, line 23 through page 9, line 8 of the original specification. In each instance, both MHC class I *and* class II are referenced.

(iv) The nature of the invention

The invention is straightforward and readily reproducible, as methods for growing cells, separating cells from culture supernatants, lysing cells, and subjecting cellular materials to differential centrifugation are not overly complex. The skilled artisan would instantly recognize how to perform these tasks to achieve the results of the inventors, and would have the means available. Generally, these tasks are performed by laboratory technicians. The Examiner has agreed with Appellants on this point in the final rejection dated January 16, 2001, paper 16, item 5.

The present invention provides evidence that MHC class I and/or II molecules are present in vesicles derived from antigen presenting cells. All that is required to prepare such vesicles is a cell expressing MHC class I and/or class II proteins and the means disclosed in the subject invention. Contrary to the Examiner's statement that "[i]f said

vesicles are identical [with the vesicles of Zitvogel et al.], that the Examiner agrees with Applicant that based on the Zitvogel article, one would know how to make and use said vesicle" (Paper 23, item 3, second paragraph), Appellant's point out that whether they are identical is not the issue; rather it is whether without undue experimentation one of skill in the art could make and use the claimed invention. One skilled in the art would instantly recognize the close similarities of the present and reference vesicles, and that the methods for preparing, testing and using them are essentially the same. The Zitvogel reference confirms that culture supernatants of antigen-presenting cells, such as the dendritic cells cited by Zitvogel (page 595, column 1, lines 5-21) and disclosed in the present invention (page 1, line 17; page 5, line 18, page 6, line 2), may be used to isolate vesicles comprising MHC class I and class II by differential ultracentrifugation of culture supernatants (Zitvogel, page 595, column 1, lines 5-21; and the present application, at page 2, line 25 through page 3, line 1). The vesicles exemplified in the present specification are derived from another type of antigen presenting cell (a lymphocyte line). Lymphocytes generally express higher levels of MHC class I proteins than any other cell in the body, thus making them even more likely to be operable and useful than dendritic cells. The vesicles isolated by Zitvogel were 60 – 90 nm in diameter (page 595, column 1, line 1) and in the present invention 60 – 80 nm in diameter (page 9, line 7). The potential uses for the vesicles discussed in the reference and disclosed in the present specification *are* identical, that being as vaccines (antitumor effects shown by Zitvogel on page 595, column 1, lines 33-35, and taught by the present specification on page 7, lines 18-19).

(v) The state of the prior art

It is generally accepted in the art that antigen presenting cells constitutively express MHC class I molecules, and that these cells also secrete vesicles. Those skilled in the art have long known that lymphocytes, an antigen presenting cell used to exemplify the present invention, generally express higher levels of MHC class I proteins than any other cell in the body. At the time the present application was filed, what the art did *not*

recognize was that vesicles derived from vesicles bearing MHC I molecules could be readily isolated from cells and be used as a means for therapeutic treatment.

(vi) The relative skill of those in the art

Cellular and molecular immunologists, medical microbiologists and physiologists, to name a few of the skilled artisans who study antigen presenting cells, are well skilled in the art of growing antigen presenting cell lines and manipulating them in the fashion disclosed in the present application to obtain the claimed vesicles. No unusual skills are required by the present invention, the skilled artisan regularly practices the techniques used, and the skilled artisan would readily recognize the methods for making and using the claimed vesicles.

(vii) The predictability or unpredictability of the art

The art of vesicle isolation and use hardly constitutes an unpredictable art. Contrary to the Examiner's statement that "in view of the lack of predictability concerning MHC class I expression on exosomes as demonstrated by the post-filing date Zitvogel reference" (advisory action Paper 18, item 2), the Zitvogel reference teaches the *predictability* of MHC class I expression on exosomes. The means to culture cells and produce vesicles is highly predictable, since the isolation procedure is straight-forward and uncomplicated, and Zitvogel et al. prepared and used vesicles in a manner similar to that disclosed in the present invention procedures (i.e., culture, differential centrifugation and Western blotting), and reproducibly found such proteins on their vesicles. The fact that Zitvogel *et al.* were unaware that such proteins were present on their exosomes prior to scrutiny, and were thus surprised by their results, does not alter the present facts, the composition of the vesicles, or the predictability of the art, any more that did the Inquisition in Rome alter the predictability of Galileo's observations. Accordingly, the Examiner's reference to the Zitvogel article in the final rejection that "it was unexpected that multivesicular late endosomes and exosomes in

dendritic cells bear MHC I class molecules" (Paper 16, page 3, lines 4-5) does not make the expression of MHC class I on exosomes unpredictable in an operative sense. Zitvogel shows that monocyte-derived dendritic cells secrete exosomes that express both MHC class I and class II. Appellants note that the thrust of the Zitvogel reference is the use of exosomes as "a novel cell-free therapeutic cancer vaccine" made possible because "endosomes and exosomes produced by dendritic cells bear MHC class I and II molecules" (page 594, column 2, lines 5-10). Tumor peptide-loaded dendritic cells (DC)-derived exosomes "induced [cytolytic T cell] priming *in vivo* and suppressed growth or induced complete regression of several established murine tumors" (page 594, column 2, lines 5-10). Zitvogel also states "[c]o-localization of MHC class I and class II molecules in DCs' endosomes was also seen in confocal microscopy" (page 595, column 1, lines 12-14), "DCs secrete a population of endosome-derived membrane vesicles which can bear both MHC I and II molecules" (page 595, column 1, lines 19-21), "(murine) [b]one marrow derived-DCs ... were analyzed by confocal and electron microscopy and found to contain multivesicular late endosomes bearing both MHC I and MHC II molecules" (page 595, column 1, lines 36-41) and "[t]hese DCs expressed low levels of ... MHC class I and II molecules, as assessed by flow cytometry analysis. Upon [lipopolysaccharide] stimulation, the expression of these molecules was upregulated. The markers expressed by the ... exosomes were characterized and quantified by electron microscopy...and Western blotting" (page 595, column 1, lines 46-51), Thus, the Zitvogel publication confirms MHC class I proteins may be found in the external membrane of endosomes, in intraluminal vesicles, at the cell surface, and in exosomes. Most significantly, Zitvogel et al. demonstrate the predictability of the art by providing methods for culturing antigen presenting cells to upregulate the expression of MHC class I proteins, and observing, characterizing and quantifying MHC class I bearing vesicles using standard, published, predictable methods.

(viii) The breadth of the claims

Appellants believe that the breadth of the claims is commensurate with the breadth of the disclosure. Independent Claim 13 is directed to an antigen presenting vesicle comprising an MHC class I protein or a functional derivative or fragment obtainable from an antigen presenting cell, for which methods of making and using are clearly related, as discussed above. Also claimed is an antigen presenting vesicle further comprising at least partially processed antigens (Claim 3) and partially processed antigens in the context of MHC class I molecules (Claim 4). Also claimed are antigen presenting vesicles using differential centrifugation of cell culture supernatants or lysates of antigen presenting cells (Claim 9) and methods for preparing said vesicles (Claims 11-12). Appellants have presented numerous examples, including differential centrifugation of supernatants and lysates of antigen presenting cells, presented in significant detail, that thoroughly exemplify the invention. Prior to the filing of the present application, there had been attempts to produce similar synthetic vesicles (e.g., liposomes) but these efforts did not prove successful (page 6, lines 7-9). Since the present invention represents the first successful attempt to produce antigen presenting vesicles comprising an MHC class I proteins, the present claims are of appropriate breadth in view of the disclosure.

C. A working definition of "vesicle" is known in the art, and fully described in the present invention.

The Examiner has stated in the first Advisory Action (Paper 18, item 2) that there is an "absence of a working definition of 'vesicle' in the instant specification", and in the second Advisory Action (Paper 23, item 3) that the Examiner is not clear whether "the term vesicle includes the endoplasmic reticulum membranes and/or Golgi membranes, where class I molecules mature and contact antigen, or additionally, even if the cell membrane could be included." Appellants respectfully point out that the term "vesicle" has long been an art-recognized term. The "On-Line Medical Dictionary definition for "vesicle" is a "closed membrane shell, derived from membranes either by a physiological process (budding) or mechanically by sonication" (entry of November 18, 1997; please

see attached copy definition). The scientific literature is replete with references to vesicles, methods for producing them, and how they may be used. For example, West et al (ref. no. 8; (1994)), describe membrane vesicles carrying newly assembled class II MHC complexes. Kleijmeer et al (ref. no. 7; (1994)) teaches MHC-II-enriched compartments with internal membrane vesicles. Riberdy et al. (ref. no. 6; (1994)) describes how class II-invariant chain complexes accumulate in large acidic vesicles. On page 2, lines 6-7, the present application refers to secreted vesicles, termed exosomes, and references two articles (ref. no. 13; Harding et al. (1984) and Pan et al. (1985) that describe how vesicles are secreted by reticulocytes and B cells (ref. nos. 5 and 6; Peters (1991) and Riberdy (1994), respectively). In a search of the scientific literature form 1985 to 1995, Appellants have found 8 articles for "vesicles" and "antigen-presenting", 19 articles comprising "vesicles" and "reticulocytes", 24 articles referring to "vesicles" and "B cells", 47 articles describing "vesicles" and "macrophages", 109 articles referencing "vesicles" and "dendritic", and 388 articles discussing "vesicles" and "antigens". These references confirm that the term "vesicle" used in the context of the present application was an art-recognized term at the time the present application was filed, and that methods for studying, making and using vesicles from antigen-presenting cells were known at that time.

Appellants also respectfully point out that the present specification characterizes the term "vesicle" in numerous locations. The specification describes exosomes as *secreted* vesicles (e.g., page 2, lines 6-7). The presence of vesicles in the disclosed preparations was verified and described using electron microscopy (page 8, line 37). The abstract defines the derivation of the claimed vesicles: "[e]xosomes are vesicles derived from MHC class II enriched compartments in antigen presenting cells. The exosomes possess MHC II and/or MHC I at their surface and possibly peptides derived from processed antigens in said MHCs." on page 1, line 24-29 (including disclosure of references 5-10 that describe methods for making and using vesicles) and page 2, lines 1 to 7. Lines 3-6 on page 6 provide a specific description for the term as used in this invention: "These vesicles preferably will contain major histocompatibility complex (MHC) I and/or II, most

preferably loaded with a peptide derived from or corresponding to an antigen which can be processed by antigen processing cells."

For all of the aforementioned reasons, the 35 U.S.C. § 112 first paragraph (enablement) rejection is erroneous and should be reversed.

(9) Conclusion

For the reasons stated above, the Examiner's rejection of Claims 2-4, 6 and 13 is erroneous. The Honorable Board is respectfully requested to reverse the Examiner's rejection of all claims on appeal and remand the application to the Examiner for allowance. Submitted herewith are three copies of Appellants' brief on appeal.

Respectfully submitted,

Dated: November 19, 2001

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Enclosures:
- Appendix A Claims on Appeal
- Appendix B Claims upon Entering Amendment after Final Rejection
- Appendix C Glossary of Terms Used in the Brief on Appeal
- Appendix D Copy of *Online Medical Dictionary* Definition of "Vesicle"

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APPENDIX A

CLAIMS ON APPEAL

2. The antigen presenting vesicle according to Claim 13, wherein said major histocompatibility complex protein is derived from MHC class I or class II.

3. The antigen presenting vesicle according to claim 13 further comprising at least partially processed antigens.

4. The vesicle according to claim 3 wherein said at least partially processed antigens are presented in the context of MHC class I proteins.

6. The antigen presenting vesicle according to claim 13, wherein said antigen presenting cell is derived from a B-lymphocyte, a Langerhans cell, a macrophage or a dendritic cell.

13. An antigen presenting vesicle free from its natural surroundings, comprising:

a membrane and a major histocompatibility complex (MHC) class I protein or a functional derivative or fragment thereof, wherein said antigen presenting vesicle is obtainable from a cell.

APPENDIX B

CLAIMS UPON ENTERING OF AMENDMENT AFTER FINAL REJECTION

3. The antigen presenting vesicle according to claim 13 further comprising at least partially processed antigens.

4. The vesicle according to claim 3 wherein said at least partially processed antigens are presented in the context of MHC class I proteins.

6. The antigen presenting vesicle according to claim 13, wherein said antigen presenting cell is derived from a B-lymphocyte, a Langerhans cell, a macrophage or a dendritic cell.

9. An antigen presenting vesicle having a membrane and a major histocompatibility complex (MHC) class I protein, obtained by the step of:

recovering a membrane-enriched fraction obtained by differential centrifugation of membrane-containing fractions of cell culture supernatants or lysates of antigen presenting cells.

11. An antigen presenting vesicle having a membrane and a major histocompatibility complex class I molecule, obtained by the step of:

recovering a 70,000 x g pellet obtained by differential centrifugation of membrane-containing fractions of cell culture media or lysates of antigen presenting cells containing MHC class I.

12. A purified antigen presenting vesicle having a membrane and a major histocompatibility complex class I molecule, obtained by the step of:

recovering a fraction having a buoyant density of 1.10 to 1.22 g/ml from a 70,000 x g pellet obtained by differential centrifugation of membrane-containing fractions of cell culture supernatants or lysates of antigen presenting cells containing MHC class I.

13. An antigen presenting vesicle free from its natural surroundings, comprising:

a membrane and a major histocompatibility complex (MHC) class I protein or a functional derivative or fragment thereof, wherein said antigen presenting vesicle is obtainable from an antigen presenting cell.

APPENDIX B

GLOSSARY OF TERMS USED IN THIS BRIEF ON APPEAL

Antigen-presenting cell

A cell that takes up antigens through endocytosis, whereafter these antigens are cleaved into peptides which are presented at the surface of said antigen presenting cells in the context of a major histocompatibility complex. By this presentation on the surface the peptides derived from the original antigen can be recognized by for instance helper T-lymphocytes, further activating the cellular immune response (in the specification, page 1, lines 6-13).

Differential centrifugation

A method of separating sub-cellular particles according to their sedimentation coefficients, which are roughly proportional to their size. Cell extracts are subjected to a succession of centrifuge runs at progressively faster rotation speeds. Large particles, such as nuclei or mitochondria, will be precipitated at relatively slow speeds; higher G forces will be required to sediment small particles, such as ribosomes (Glossary of Biotechnology and Genetic Engineering, Food and Agriculture Organization of the United Nations; http://www.fao.org/DOCREP/003/X3910E/X3910E07.htm)

Exosome

Cellular vesicles such as endosomes "that could be released outside the cell following direct fusion of the external membrane of the endosome with plasma membrane" (Zitvogel *et al.*, *supra*).

Immunoprecipitation

The phenomenon of aggregation of sensitized antigen upon addition of specific antibody (precipitin) to antigen in solution (Stedman's Medical Dictionary, 26th Ed., 1995. Williams and Wilkins).

Lipopolysaccharide

A compound or complex of lipid and carbohydrate; the 1. (endotoxin) released from the cell walls of Gram-negative organisms that produces septic shock solution (Stedman's Medical Dictionary, 26th Ed., 1995. Williams and Wilkins).

Lymphocyte

A white blood cell formed in lymphatic tissue throughout the body;

B-lymphocytes: an immunologically important lymphocyte [that is] responsible for the production of immunoglobulins;

T-lymphocytes: a thymocyte-derived lymphocyte of immunological importance that...is responsible for cell-mediated immunity;

(Stedman's Medical Dictionary, 26th Ed., 1995. Williams and Wilkins).

MIIC

A lysosome-like, MHC-class II-enriched compartment which contains characteristic membrane vesicles and concentrically arranged membrane sheets (in the specification, page 1, lines 26-28).

Major histocompatibility complex (MHC) proteins

The products of genes grouped together on a chromosome that determine whether transplanted tissues will be accepted (if from an individual with the same MHC) or rejected. In the human, they are known as *human leucocyte antigen molecules* (*HLAs*). MHC gene products ... consist

of two types: *class I* ...[and] *class II* (Glick, *Glossary of Biochemistry and Molecular Biology*, 1996, Portland Press; Elliott, T., *et al.* (1993) *Curr. Biol.* **3**, 854-866; Gumperz, J.E. *et al.* (1995) Nature (London) **378**, 245-248).

Vesicle

A "closed membrane shell, derived from membranes either by a physiological process (budding) or mechanically by sonication" (On-Line Medical Dictionary, November 18, 1997, http://www.graylab.ac.uk/cgi-bin/omd?query=vesicle&action=Search+OMD)

Western blotting (immunoblotting)

Process by which antigens can be separated by electrophoresis and allowed to adhere onto nitrocellulose sheets where they bind nonspecifically and then are subsequently identified by staining with appropriately labeled antibodies (Stedman's Medical Dictionary, 26th Ed., 1995. Williams and Wilkins).

Appendix D

vesicle

<pathology> A closed membrane shell, derived from membranes either by a physiological process (budding) or mechanically by sonication.

Vesicles of dimensions in excess of 50nm are believed to be important in intracellular transport processes.

See: coated vesicles.

(18 Nov 1997)

Previous: vesbium, vesica, vesical, vesicant, vesicate, vesication, vesicatory
Next: vesico-, vesicoprostatic, vesico-ureteral reflux